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EFFECT OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF HYDROGEN AND CARBON MONOXIDE BACTERIA

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EFFECT OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF HYDROGEN AND CARBON MONOXIDE BACTERIA[1]

By G. W. Jones[2] and G. S. Scott[3]

It has been shown that hydrogen and carbon monoxide may be oxidized to water and carbon dioxide by bacterial action,[4] [5] and that the bacteria responsible for the oxidation of hydrogen are fairly widely distributed in nature.[6]

The activity of these bacteria is of special importance in connection with mine fires, inasmuch as it is the usual practice to assume that a fire is out when carbon monoxide is no longer present in the fire area. That such an assumption may be misleading and at times dangerous is now evident.

Samples of mine waters from anthracite workings have shown that carbon monoxide and hydrogen bacteria usually are present, while most of the samples of mine waters tested to date from bituminous mines have not shown such activity. It was thought that the difference might be due to greater acidity of bituminous mine waters, and knowledge of the effect of acidity and alkalinity on the growth of these organisms seemed desirable.

Two series of tests were made - a preliminary series to establish the approximate pH limits between which these bacteria showed activity and a second series to define the limiting values with greater precision.

In the preliminary tests small reaction vessels, previously described[4], were used. A gas mixture of the following composition was employed to study the activity:

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[1] The Bureau of Mines will welcome reprinting of this paper provided the following footnote acknowledgment is used: "Reprinted from Bureau of Mines Information Circular 7133."

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[4] Jones, G. W., and Scott, G. S., Carbon Monoxide in Underground Atmospheres: Ind. Eng. Chem., vol. 31, 1939, pp. 775-778.

[5] Jones, G. W., and Scott, G. S., Oxidation of Carbon Monoxide and Hydrogen by Bacteria: Bureau of Mines Rept. of Investigations 3466, 1939, 10 pp.

[6] Lee, S. B., and Umbreit, W. W., Biological Oxidation of Molecular Hydrogen; Centralblatt Bakt., vol. 101, pt. 2, 1940, pp. 354-363.

Constituents	Percent by volume
Carbon dioxide..	1.6
Oxygen.........	12.2
Hydrogen........	7.6
Carbon monoxide.	15.6
Nitrogen........	63.0

Inoculation was conducted as follows:

The liquid from a previous test that had proved especially active in respect to hydrogen and carbon monoxide oxidation and that had a pH value of 6.3 was used. Sulfuric acid was added to one portion until the pH value equaled 5.30. A test sample was prepared having equal parts of this liquid and the gas mentioned above. Sulfuric acid was then added to another portion of the liquid until the pH value was 3.85 and a second test sample was prepared. A similar procedure was followed on the alkaline side with sodium hydroxide.

After standing 36 days at the temperature of the laboratory, the gas was removed and analyzed. The results are given in table 1.

TABLE 1

Composition of the original gas mixture

pH	CO_2	O_2	H_2	CO	N_2
	1.6	12.2	7.6	15.6	63.0

Composition of final gas mixtures at end of 36 days

2.80	6.2	4.7	0.6	15.0	73.5
3.85	.8	19.9	-	-	-*
5.30	13.2	.4	.4	0.3	85.7
6.60	9.3	.4	0	0	90.3
8.35	8.4	1.4	0	0	90.2
9.00	9.8	.7	0	0	89.5
9.90	5.9	.7	1.7	4.8	86.9

*Obviously a leak existed in this assembly.

Inspection of the results indicated that the pH limits for the growth of these organisms lie approximately between 3.0 and 10.0.

During the interim between these and the second series of tests, pH determinations were made on samples being tested for presence of hydrogen and carbon monoxide bacteria. The results of the tests are tabulated below:

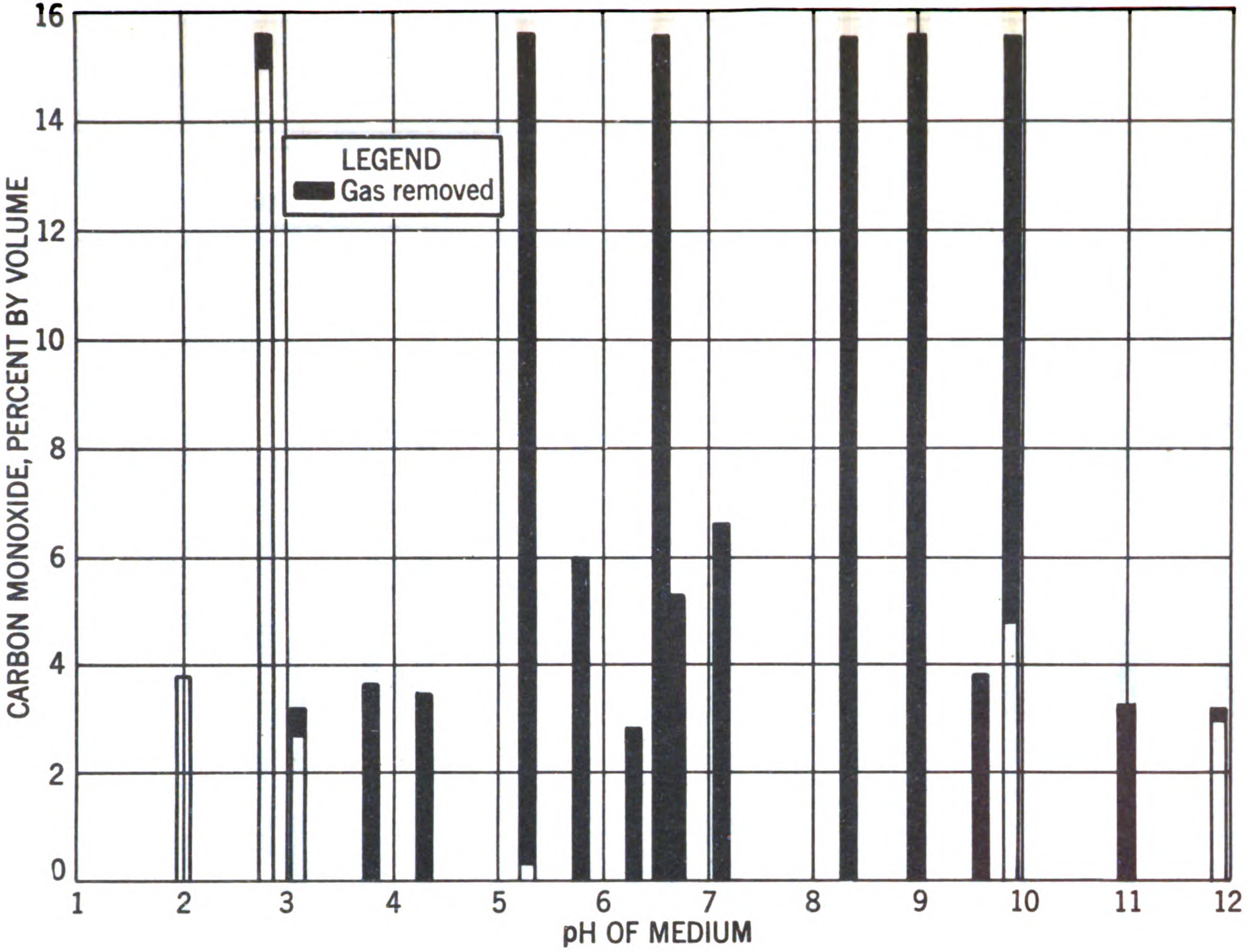

Figure I.—Effect of pH on growth of carbon monoxide bacteria.

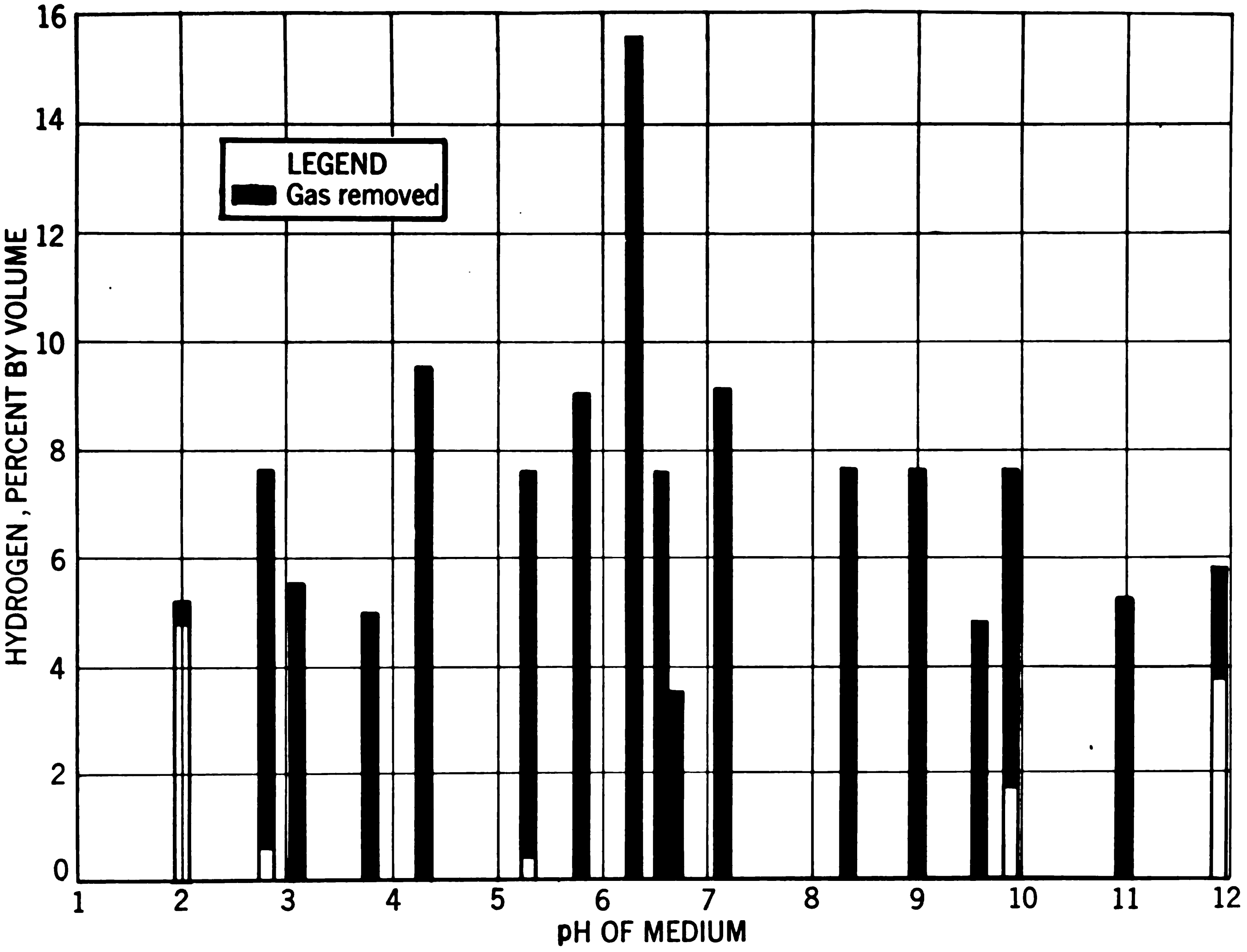

Figure 2.—Effect of pH on growth of hydrogen bacteria.

TABLE 2. - Composition of initial and final gas mixtures, miscellaneous samples

Source and pH	Reaction time, days	Composition, percent by volume				
		CO_2	O_2	H_2	CO	N_2
1. Garden soil no. 1 and tap water, pH 7.15	0	0	17.6	9.1	6.6	66.7
	35	8.0	11.7	0	0	80.3
2. Garden soil no. 2 and tap water, pH 6.65	0	0	19.0	3.5	5.3	72.2
	19	5.8	15.9	0	0	78.3
3. Spring water, pH 5.8	0	0	17.8	9.0	6.0	67.2
	22	17.2	0.2	0	0	82.6
4. Brook water, pH 4.3	0	0	18.3	9.5	3.4	68.8
	33	13.4	5.5	0	0	81.1

Sponges were used to accelerate the reactions, and some of the carbon dioxide shown in the analyses, especially Nos. 3 and 4, made at the end of the tests apparently resulted from oxidation of the sponges. The results of these tests are given in figures 1 and 2.

The final series of tests was made out in 5-gallon bottles, previously described.[4/]

The bottles, each containing 2 liters of tap water and a small piece of sponge to supply mineral matter, were sterilized at 15 pounds pressure in an autoclave. The glass-tube and stopcock assemblies likewise were sterilized. The purpose of the sterilization was to destroy any bacteria that might be present before the active specimens were introduced.

After sterilization, measured amounts of hydrogen and carbon monoxide were added to each bottle and the gaseous atmosphere was analyzed to make certain that the initial composition of the gas was approximately the same for all bottles.

A leveling bottle was then connected to the side tube and some of the liquid was removed and hydrochloric acid or potassium hydroxide solution added to the bottles. The bottles were then shaken and some liquid withdrawn, treated, and returned to the bottle until a uniform distribution of the acid or alkali was obtained. In this manner the pH values of 6 bottles were adjusted to 2.0, 3.1, 318, 9.6, 11.0, and 11.9.

The bacteria used for inoculation consisted of the scum formed on the surface of sample 1 (table 2). This was then stirred vigorously to break up the larger pieces, and measured quantities of this suspended scum were added to the 5-gallon bottles.

Temperature, barometric pressure, and manometer readings were taken regularly and the gaseous atmosphere was analyzed at frequent intervals. The results are given in table 3.

The reactions taking place have been discussed.[4]

TABLE 3. - Effect of acid and alkaline mediums on the activity of hydrogen and carbon monoxide bacteria

Test No.	Duration of test, days	pH	Relative volume	Composition of gaseous atmosphere, percent by volume				
				CO_2	O_2	H_2	CO	N_2
1	0	2.0	1.000	0.0	18.5	5.2	3.8	72.5
	16	-	.994	.6	18.3	5.2	3.8	72.1
	34	2.2	.992	.9	18.0	4.8	3.8	72.5
2	0	3.1	1.000	0.0	18.8	5.5	3.2	72.5
	13	-	.946	1.1	16.4	2.7	3.4	76.4
	20	-	.905	1.5	15.7	.0	3.6	79.2
	31	7.2	.894	3.6	13.5	.0	3.0	79.9
3	0	3.8	1.000	.0	18.8	4.9	3.7	72.6
	13	-	.887	6.4	11.8	.0	.8	81.0
	20	-	.887	8.3	9.8	.0	.0	81.9
	31	7.0	.875	9.5	8.6	.0	.0	81.9
4	0	9.6	1.000	.0	18.8	4.8	3.8	72.6
	6	-	.909	2.8	15.6	.2	1.9	79.5
	13	-	.880	7.2	11.5	.0	.0	81.3
	31	7.0	.872	8.6	9.3	.0	.0	82.1
5	0	11.0	1.000	.0	19.0	5.2	3.3	72.5
	8	-	.946	.5	17.5	1.6	2.8	77.6
	13	-	.886	3.5	15.5	.0	.2	80.8
	34	7.1	.866	4.9	13.5	.0	.0	81.6
6	0	11.9	1.000	.0	18.8	5.8	3.2	72.2
	16	-	.978	.0	18.4	5.8	3.1	72.7
	34	11.5	.938	.0	18.0	3.9	3.2	74.9

On examining table 3, it will be observed that the pH values approach the neutral point in those cases where oxidation took place. A number of questions arose concerning these changes in pH values. Did the change take place gradually throughout the tests, or rather suddenly at some stage of the process? Did the hydrogen and carbon monoxide disappear during or after the pH change?

To answer these questions, two tests were repeated, and regular measurements of the pH values were made by removing small portions of the liquid from the bottles and measuring the pH value with a Coleman electric pH meter. The results of these two tests are shown graphically in figures 3 and 4.

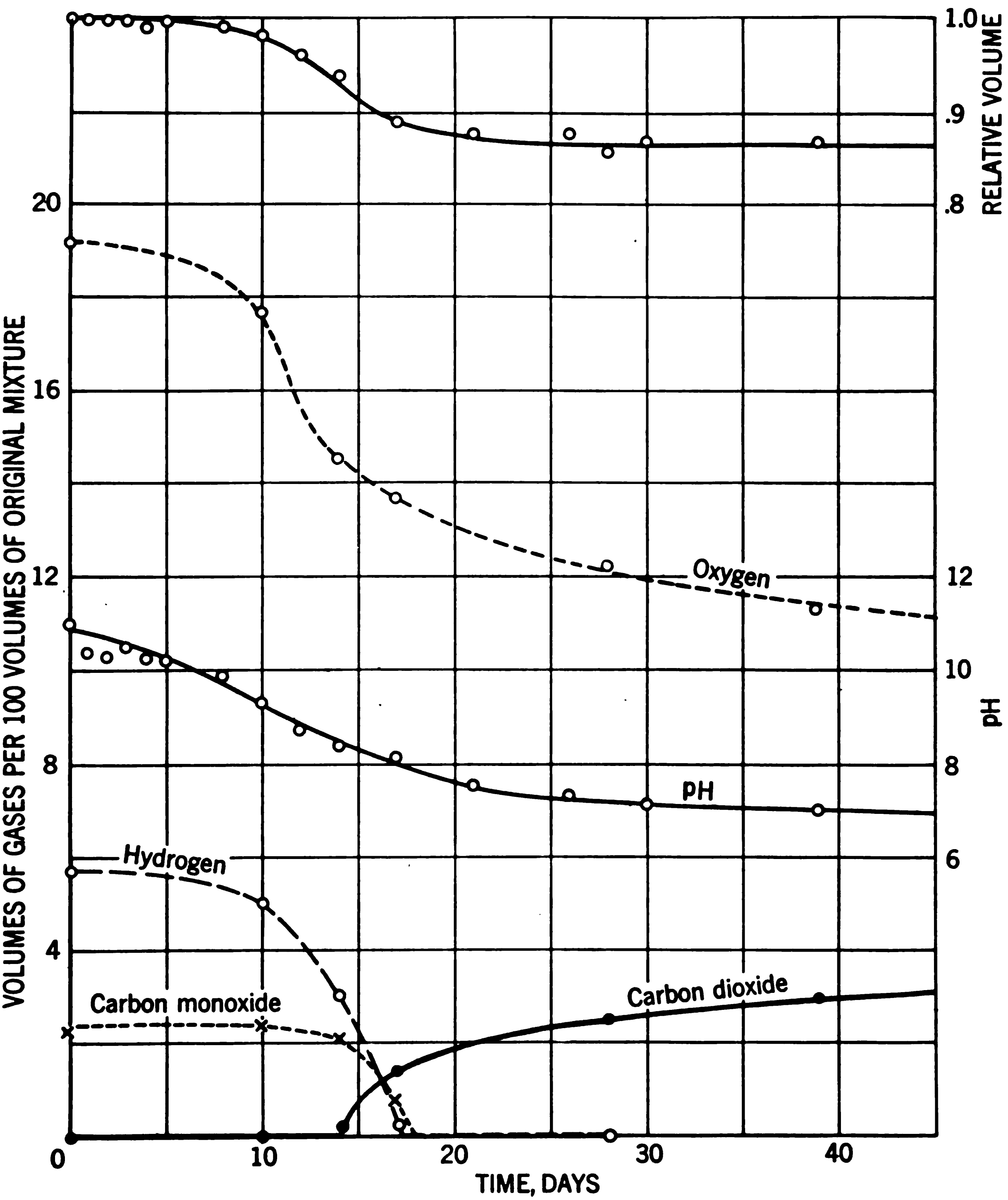

Figure 3.—Change of pH and composition of gases with time. Alkaline medium.

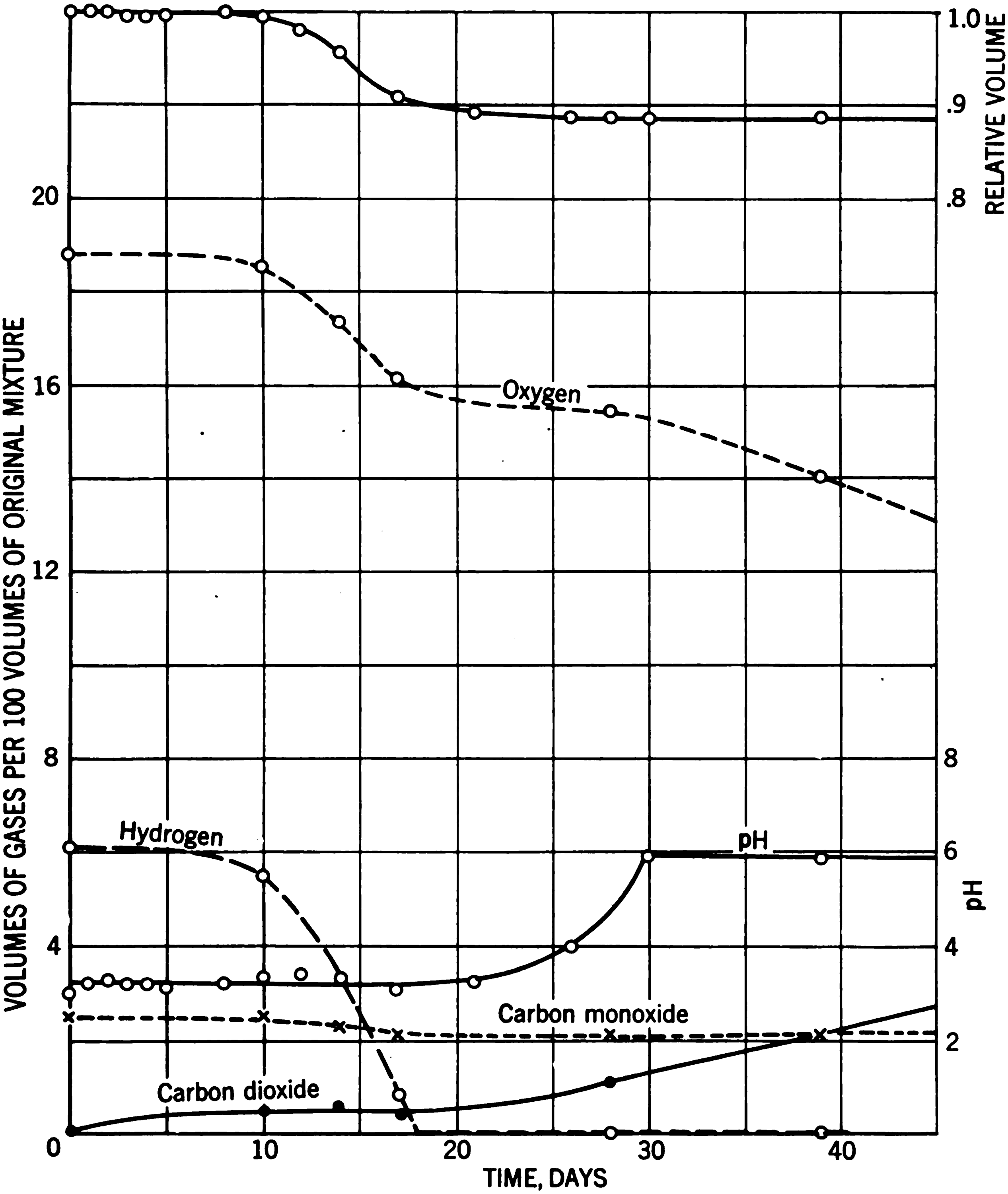

Figure 4.—Change of pH and composition of gases with time. Acid medium.

From the alkaline side (fig. 3), it appears that the bacterial oxidation and the pH values change simultaneously. From the acid side (fig. 4), however, it will be observed that the hydrogen has disappeared before any appreciable change in pH has occurred. The carbon monoxide, on the other hand, shows but little change.

CONCLUSIONS

The tests reported indicate that the disappearance of carbon monoxide from a gaseous atmosphere as a result of bacterial action can take place when the initial pH value of the active medium lies between 3.8 and 11.0 while the limits for hydrogen under the same conditions are 2.8 and about 11.5.

The pH value in mediums that react tends to approach the neutral point as the oxidation of hydrogen and carbon monoxide proceeds toward completion.